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FY 1994 ANNUAL REPORT

Veterinary Resources Program

National Center for Research Resources

National Institutes of Health

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VETERINARY RESOURCES PROGRAM
NATIONAL CENTER FOR RESEARCH RESOURCES
NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT FOR FY 1994

ROGER D. ESTEP, D.V.M.
ACTING DIRECTOR

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VETERINARY RESOURCES PROGRAM PROJECTS (Z01 RR)

NO.	TITLE	INVESTIGATOR	PAGE
00001-24	Animal Model Development	C.T. Hansen	1
00077-10	Development of an Embryo Cryopreservation Program In Laboratory Animals	P.M. Schmidt	3
00087-05	Serum Cholesterol and Triglyceride values of the NIH WHHL Rabbit Colony	D.E. Barnard	5
00090-05	Characterization of the WHHL Rabbit Lipoprotein Phenotype	D.E. Barnard	7
00095-04	Pathogenesis of Ophidian Paramyxovirus Infection in Brown Tree Snakes	E. Woo	9
10400-03	Idiopathic Hepatitis in the N:NIH(S)-B Mouse	J. A. Burris	11
10432-02	Pathogenicity of CAR Bacillus (Mouse Origin) in Immunodeficient Strains of Mice	M. A. Eckhaus	13
10433-02	Detection and Diagnosis of Mycobacterial Infection in Nonhuman Primates	A. A. Azadegan	15
10434-02	Development of Embryo Cryopreservation Procedures for Laboratory Rats	W.F. Rall	17
10435-02	A New Pathogenic Bacterium of the Haemophilus/Pasteurella Group in Hamsters.	J. A. Burris	19
10436-02	DNA Fingerprinting Inbred Rats and Mice	M. G. Mandel	21
10437-02	Infection with *Mycoplasma pulmonis* in SCID and BALB/C Mice	G. F. Miller	23

Table of Contents (continued)

10438-02	Hemosiderosis in Marmosets: A Potential Animal Model for Human Hemochromatosis	G. F. Miller	25
10439-02	Effects of Environment on Socially-housed Rhesus Monkey Reactions to Novel Objects	K.A.L. Bayne	27
10442-01	Hepatic Hemosiderosis in Marmosets	G.F. Miller	29
10453-01	Comparative Behavior analysis of Vertical Cage Space for Grouped Housed Rhesus	K.A.L. Bayne	31

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-00001-24 VR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Animal Model Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Carl T. Hansen, Ph.D.	Geneticist	GRS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Genetic Resource Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is designed to respond to the continuing requirement by the research community for small animal models of human diseases. Since the inception of this project, rat models have been developed for the study of rheumatoid arthritis, insulin independent diabetes mellitus, chemical addictions, as well as behavioral disorders. In the mouse, the emphasis has been on developing models for the study of diseases in which the immune system plays a critical role.

A related component of this project has been the study of those aspects of the reproductive cycle in lagomorphs and rodents which have a significant impact upon their reproductive efficiency. These studies have greatly facilitated the development and maintenance of these animal models.

Objective:

To develop and maintain animal models of human diseases for use by the biomedical research community.

Methodology:

The primary means of developing these models is by a program of selective breeding. That is, only those individuals exhibiting the trait of interest are selected to reproduce and to be parents for the succeeding generation. The assumption for undertaking a specific project is that a trait is of value to the biomedical research community and it is at least partially genetically determined.

Major Findings:

Results from a project of this nature are incremental in nature since the trait(s) of interest have a complex genetic basis. Thus, the findings during a particular year usually represent only a phase of a long-term project. The study to elucidate the role of the x-linked Immune Defect (Xid) in the development of a mouse AIDS-like syndrome (MAIDS) continues. It was originally noted that, unlike "normal" littermates, C57BL/6N mice carrying the Xid mutation did not develop the MAIDS syndrome when exposed to the appropriate viruses. More recently, two additional strains, P/N and SJL/N have been added to this study.

Several rat models continue to be developed. DSS rats were selective bred for sensitivity to a high salt diet. This diet created a maligant hypertension with numerous vascular complications. The condition was fatal. More recently it has been discovered that animals homozygous for another mutation, the corpulent gene, exhibit the same classical symptoms of vascular involvement when fed a high sucrose diet, but fail to develop the elevated blood pressure. Projects in more prelimenary stages include an attempt to map those gene(s) which contribute to the susceptibility or resistance to rheumatioid arthritis in the rat. Studies analyzing the relationship between the endocrine and immune systems have been initiated using the F344/N and LEW/SsN rat strains.

Proposed Course:

Continuation.

Publications:

Cash JM, Remmers EF, Goldmuntz EA, Crofford LJ, Zha H, Hansen CT, Wilder R. Genetic mapping of the Athymic Nude (RNU) locus to a region on Chromosome 10. Mammalian Genome 1993; 4:37-42.

Caspi RR, Chan C, Fujino F, Najafian S, Grover S, Hansen CT, Wilder RL. Recruitment of antigen-nonspecific cells play a pivotal role in the pathogensis a T-cell mediated organ-specific autoimmune disease, Experimental Autoimmune Uveoretinitis. Journal of Neuroimmumology 1993; 477:177-189.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-00077-10 VR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of an Embryo Cryopreservation Program in Laboratory Animals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Patricia M. Schmidt, M.S.	Physiologist	GRS, SSB, VRP, NCRR
Carl T. Hansen, Ph.D.	Geneticist	GRS, SSB, VRP, NCRR
Xi Lin, D.D.S.	Fogerty Fellow	GRS, SSB, VRP, NCRR
William F. Rall, Ph.D.	NRC Senior Research Associate	GRS, SSB, VRP, NCRR
David E. Wildt, Ph.D.	Guest Researcher	LVC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Genetic Resource Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.6	2.7	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The NIH Animal Genetic Resource (NIHAGR) maintains a large number of animal species and many invaluable genotypes within a species used as models in biomedical research. To protect this germ plasm, an embryo bank was established as an economical means to indefinitely preserve these stocks and strains of rodents and rabbits. Cryopreservation of embryos has other benefits for genotype management such as safeguarding against loss of germ plasm from disease, reproductive failure, natural catastrophe or genetic contamination. The cryobank minimizes changes due to genetic drift by providing a resource from which to periodically rederive defined genotypes for restandardization of foundation breeding colonies. As a recognized repository for laboratory animal species, the NIHAGR routinely provides breeding nuclei upon request throughout the biomedical research community. Rather than shipping live animals, cryopreserved embryos represent a simpler, more humane and easier method to transport requested germ plasm worldwide. Another important impact of embryo handling, cryopreservation, and transfer procedures is to eliminate the vertical transmission of some murine diseases.

Objective:

The objective is to study the development and cryobiology of embryos and gametes from laboratory animal species for the purpose of improving long-term cryostorage of valuable genetic material managed within the NIH Animal Genetic Resource (NIHAGR).

Methodology:

Pre-implantation stage embryos were collected from hormonally-treated or natural estrus females following euthanasia or at surgery. Embryos were frozen or vitrified in medium containing cryoprotectant. Following storage in liquid nitrogen (-196°C), straws containing embryos were warmed and the cryoprotectant removed using a sucrose dilution procedure. Diluted embryos were assessed *in vitro* or *in vivo*. Embryo stage of development and quality were observed daily to determine *in vitro* survival for embryos in culture. *In vivo* evaluation involved surgical transfer of embryos into synchronized, pseudopregnant recipients. Recipients were monitored for pregnancy and allowed to go to term. If embryos were intended as additions to the program cryobank, individual animal pedigree records were maintained on the Genetic Resource Section animal database system.

Major Findings:

Since 1984, approximately 269,095 mouse embryos were collected. Of these, approximately 91,145 were permanently banked and the remainder were used in comparative biological studies. To date, approximately 1,000 non-pedigreed embryos have been stored from each of 35 NIHAGR genotypes. During FY 1994, over 47,023 embryos were collected from 116 mouse strains. All embryos banked this year were collected from pedigreed donors. More than 18,104 embryos were cryopreserved including 16,506 embryos from 95 genotypes that were added to the embryo bank. The number of pedigreed embryos banked currently totals approximately 30,873.

This year mouse cryobanking activities showed a marked increase. Dedicated support in the barrier animal rooms administered hormonal treatments to pedigreed females, paired them with the genetically appropriate male and then sent the donors to the laboratory. These procedures allowed Embryo Cryopreservation Program personnel to handle additional animals. Compared to FY 1993, seven times more pedigreed mouse embryos were collected. A six-fold increase in the number of embryos banked this year resulted. Additionally, twice as many genotypes were included in the project than last year. The most significant increase was in the total number of pedigreed embryos banked. The FY 1994 total (16,506), was slightly more than the total for the previous 4 years combined (15,502).

During FY 1994, pedigreed embryo banking of outbred stocks (N:NIH and N:GP) was initiated.

Significance:

The concerns surrounding conservation of valuable germ plasm are just developing, historically these problems have not been at the forefront of resource management and biomedical research issues. Because of the wide range of species and genotypes representing several genetic backgrounds maintained in the NIHAGR, this is an important issue. Gamete cryopreservation presents a convenient and economical method to maintain the gene pool while limiting the impact of genetic drift on these colonies.

The data collected in the last 12 months for the cryobanking project is unique. When analyzed, this will provide an indication of whether there is a seasonal effect in the response of these mouse genotypes to hormonal treatment. If season of the year impacts negatively on embryo collection rates, it may be necessary to consider this factor when attempting to maximize the efficiency of embryo banking. The data also provides an estimate of maximum efficiency for this project with currently available resources.

Proposed Course:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-00087-05 VR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Serum Cholesterol and Triglyceride Values of the NCRR WHHL Rabbit Colony

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dennis E. Barnard, M.S.	Biologist	LSS, SSB, VRP, NCRR
Donna Jarrell, D.V.M.	Veterinarian	GRS, SSB, VRP, NCRR
Carl T. Hansen, Ph.D.	Geneticist	GRS, SSB, VRP, NCRR
Myrna Mandel, Ph.D.	Molecular Biologist	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Watanabe heritable hyperlipidemic (WHHL) rabbit breeding colony maintained by the Veterinary Resources Program, National Center for Research Resources, is a source of this animal model for investigators involved in human familial hypercholesterolemia research. The objective of this project is to characterize the serum cholesterol and triglyceride values in the rabbit colony, and evaluate the influence of genotype on cholesterol and triglyceride concentrations of the NIH WHHL rabbits. Blood was collected from WHHL rabbits at eight weeks, three months, seven months, and twelve months of age. The serums were analyzed for lipoprotein cholesterol and triglycerides. Total serum cholesterol values range from 310 to 1251 mg/dl with a mean of 828 mg/dl. The low density lipoprotein (LDL) cholesterol values range from 70 to 1185 mg/dl with a mean of 630 mg/dl. Serum triglyceride values range from 107 to 959 mg/dl with a mean of 394 mg/dl. There appears to be a correlation between serum triglyceride concentration and the sex of the rabbit. The mean male and female triglyceride concentrations were 510 mg/dl and 309 mg/dl, respectively.

Objective:

The objective of this project is to characterize the serum cholesterol and triglyceride values, and evaluate the influence of genotype on cholesterol and triglyceride concentrations. Analyses for lipoprotein cholesterol and triglyceride concentrations are being reimplemented this year to enhance colony management.

Methodology:

Blood was collected from WHHL rabbits at eight weeks, three months, seven months, and twelve months of age. The serums were analyzed for lipoprotein cholesterol and triglycerides. Blood will be collected from WHHL rabbits for the next year, and the lipoprotein cholesterol and triglyceride values will be compared with the results from the previous analyses. A computer program has been developed to track pedigrees of the WHHL rabbits, making it possible to evaluate the influence of genotype on cholesterol and triglyceride values.

Major Findings:

Total serum cholesterol values for the NIH WHHL rabbit colony ranged from 310 to 1251 mg/dl with a mean of 828 mg/dl. The LDL cholesterol values ranged from 70 to 1185 mg/dl with a mean of 630 mg/dl. Thus, 80% of the total cholesterol was composed of LDL cholesterol. There was a substantial decrease from 885 to 576 mg/dl in serum total cholesterol between the ages of eight weeks and one year--the most significant decrease occurring between 15 and 25 weeks. Differences in total cholesterol concentrations were not related to sex. Serum triglyceride values ranged from 107 to 959 mg/dl with a mean of 394 mg/dl. There was an initial increase in triglyceride concentration from 367 to 565 mg/dl between two months and three months of age. The serum triglyceride concentration was influenced by the sex of the rabbit. The mean male and female serum triglyceride concentrations were 510 mg/dl and 309 mg/dl, respectively.

Significance:

It appears that serum cholesterol and triglyceride values are influenced by age and sex, respectively. This base line data will be important to investigators designing studies involving the WHHL rabbit.

Proposed Course:

Completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-00090-05 VR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of the WHHL Rabbit Lipoprotein Phenotypes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dennis E. Barnard, M.S.	Biologist	LSS, SSB, VRP, NCRR
Donna Jarrell, D.V.M.	Veterinarian	GRS, SSB, VRP, NCRR
Myrna Mandell, Ph.D.	Molecular Biologist	LSS, SSB, VRP, NCRR
Carl T. Hansen, Ph.D.	Geneticist	GRS, SSB, VRP, NCRR
James Crowell	Geneticist	SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Veterinary Resources Program, National Center for Research Resources maintains a Watanabe Heritable hyperlipidemic (WHHL) rabbit breeding colony as a source of these rabbits for the biomedical research community. It has been reported that there are two types of WHHL rabbits--those with early development and high incidence of coronary atherosclerosis (type 1) and those with late development and low incidence of coronary atherosclerosis (type 2). It has also been shown that either type of rabbit can be selectively bred to produce progeny exhibiting the phenotype of interest. Recently, it was reported that these two types of WHHL rabbits can be differentiated by their lipoprotein phenotypes.

Rabbits were fasted overnight and blood was collected in EDTA vacutainers the next morning. Agarose gel electrophoresis was used to identify the lipoprotein phenotypes of 14 rabbits from the NCRR WHHL rabbit colony. The results showed two distinct lipoprotein phenotypes: one with an increased density of beta-lipoprotein and another with a beta-doublet. These phenotypes are similar to those reported previously for the two types of WHHL rabbits. The serum triglyceride concentration of the rabbits with the beta-doublet phenotype, 498±62 mg/dl, was significantly higher than that of the other rabbits, 260±65 mg/dl. However, the total serum cholesterol concentrations were not significantly different.

Characterization of the NCRR WHHL rabbits as to lipoprotein phenotype will be of value to investigators using them in research.

Objectives:

The purpose of this study is to characterize the lipoprotein phenotypes in the NCRR WHHL rabbit colony so that the two types of WHHL rabbits can be identified, and determine if an apoprotein E defect is present in the WHHL rabbits.

Methodology:

Rabbits were fasted overnight and blood was collected in EDTA vacutainers the next morning. Agarose gel electrophoresis was used to identify the lipoprotein phenotypes. Cholesterol and triglyceride concentrations were determined by enzymatic assays.

Major Findings:

Two lipoprotein phenotypes were identified: one with an increased density of beta-lipoprotein and the other with a beta-doublet. These phenotypes are similar to those reported in the two types of WHHL rabbits. The triglyceride concentration of the rabbits with the beta-doublet lipoprotein phenotype was significantly greater, 498±62 mg/dl, than that of the other rabbits, 260±65 mg/dl. However, the total serum cholesterol concentrations were not significantly different. The results of this study indicate that the WHHL rabbits with the beta-doublet phenotype and the type 1 WHHL rabbits have similar VLDL characteristics.

Significance:

The two lipoprotein phenotypes indicate that both the rabbit strain with the high incidence of coronary atherosclerosis and those with a low incidence may be present in the colony. Determination of the lipoprotein phenotypes will enable the Genetic Resource Section to selectively breed the rabbits, providing a more defined WHHL rabbit. A comparison of the beta-doublet lipoprotein phenotype observed in NCRR WHHL rabbits and the phenotype reported for type 1 WHHL rabbits with human lipoprotein phenotypes indicates there is a resemblance to type III hyperlipoproteinemia. Type III hyperlipoproteinemia is the result of an apoprotein E defect. Therefore, some WHHL rabbits may have an apoprotein E defect as well as an LDL receptor defect. This would explain the high incidence of coronary atherosclerosis in the type 1 WHHL rabbits.

Proposed Course:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-RR-00095-04 VR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Ophidian Paramyxovirus Infection in Brown Tree Snakes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Elaine W. Lamirande, B.S.	Biologist	LSS, SSB, VRP, NCRR
Thomas W. Spencer, B.S.	Microbiologist	LSS, SSB, VRP, NCRR
Jennie W. Owens, M.S.	Biologist	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

Department of Pathology, National Zoological Park, Smithsonian Institution

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is an ongoing project to determine the susceptibility of brown tree snakes (Boiga irregularis) to ophidian paramyxovirus infection and to study the pathogenesis of viral infections with this important group of snake pathogens. Two newly-isolated strains of ophidian paramyxovirus are currently being studied. Following inoculation with these viruses, brown tree snakes have become clinically ill and 50% have died. Paramyxoviruses have been isolated from infected snakes and propagated in cell culture; the viruses have also been identified via transmission electron microscopy. Snake inoculation trials are continuing at the National Zoo and the Veterinary Resources Program is continuing virus isolation and culture research.

PHS 6040 (Rev. 5/92)

Objectives:

To experimentally inoculate the brown tree snake (Boiga irregularis) with ophidian paramyxovirus and compare/contrast the pathogenesis of infections with different strains of paramyxovirus in this species of snake.

Methodology:

Brown tree snakes were inoculated intertracheally and per os with ophidian viruses in three separate trials. In the first trial, a paramyxovirus originally isolated from an outbreak of fatal disease in the snake colleceion at the Baltimore Zoo was used. The second virus was a strain of paramyxovirus newly-isolated from the tissues of an Eastern king snake (Lampropeltis getulus). The third virus was a retrovirus isolated from an ATCC Russell's viper spleen cell line.

The snakes are individually housed in Horsfall isolation units and are closely monitored for signs of illness. Blood samples are collected periodically and assayed for changes in the hemograms and serum biochemical parameters. Sera are also used for the development of assays to detect host antiviral antibodies. Complete necropsies are performed on any snakes that die and tissue samples are collected to histopathology, virus isolation, and electron microscopy.

Major Findings:

The different strains of paramyxovirus have demonstrated varying cytopathic effects in cell culture.

All snakes inoculated with the two strains of ophidian paramyxovirus have become clinically ill with respiratory disease. In each trail, 50% of inoculated snakes have died and have had histologic lesions indicative of viral pneumonia.

Paramyxovirus has been isolated from brown tree snake tissues through cell culture and identified by electron microscopy.

Significance:

Snakes and other reptiles are commonly kept in captivity, and their use as laboratory animals is increasing. Relatively little is known about the diseases of these animals. This study represents the first known attempts to experimentally transmit ophidian paramyxovirus in brown tree snakes. Two strains of paramyxovirus have caused significant disease in brown tree snakes, suggesting that further studies with these and/or similiar viruses are warranted to discover potential biologic control agents for this snake species.

Proposed Course:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10400-03 VR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Idiopathic Hepatitis in the N:NIH(S)-B Mouse

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jennifer A. Burris, D.V.M.	Senior Veterinary Pathologist	LSS, SSB, VRP, NCRR
Michael A. Eckhaus, V.M.D.	Pathology Unit Chief	LSS, SSB, VRP, NCRR
Dennis E. Bernard, M.S.	Biologist	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, LSS

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.04	0.02	0.02

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

All N:NIH(S)-B mice have multiple foci of hepatic necrosis and inflammation. The cause of hepatitis is unknown. This mouse strain was developed at the NIH Animal Genetic Resource for use as foster mothers in the barrier facility. All mice appear clinically normal, but have macroscopic, microscopic, and biochemical liver abnormalities. Studies are in progress to determine the etiology and to explore the pathogenesis of hepatitis in this strain. Vitamin A is being examined as a possible etiology or co-factor. Mouse strains closely related to the N:NIH(S)-B are being examined for hepatic abnormalities. Hepatitis in this strain may serve as a useful model to study the pathogenesis of hepatitis in animals and humans.

Objectives:

To determine the etiology and study the pathogenesis of idiopathic mouse hepatitis.

Methodology:

Mice are housed in a conventional facility in filter top cages. They are autopsied at 9, 10, and 12 weeks and at 12, 18, and 24 months of age. Gross and histologic lesions are assessed. SCID mice were orally and parenterally inoculated with fresh homogenized affected livers and then were autopsied at 1 week, 2 weeks, and 3 weeks post-inoculation. Serology, TEM, serum chemistries, liver cultures, special histologic stains, and immunocytochemistry have been employed in our studies. Mice on three special diets (low vitamin A, normal vitamin A, and high vitamin A) are paired and progeny examined at 10 weeks of age for lesions.

Major Findings:

Chronic and active hepatitis has been detected in all ages examined from 2 weeks to 2 years of age. There are a range of microscopic lesions, many of which can be found in any individual mouse, and all of which are found within any age group. An infectious etiology has been ruled out based on a variety of examinations and experiments to detect an agent including serology, bacteriology, transmission electron microscopy, light microscopy with special stains, immunocytochemistry, and transmission studies. Infection with the recently discovered murine hepatic Helicobacter sp. was ruled out. Results suggest that high levels of vitamin A in the diet reduces the incidence and severity of hepatitis. The closely related N:NIH Swiss mouse has a comparable high incidence and severity of idiopathic hepatitis; however, closely related CR:NIH Swiss mice have a low incidence and severity. This suggests a possible role for as yet undetermined environmental or genetic factors in the pathogenesis of the disease. Idiopathic hepatitis has also been detected in numerous other strains of mouse at a very low incidence and severity.

Significance:

The cause and pathogenesis of idiopathic mouse hepatitis remains unknown. This disease has been detected in numerous strains of mice. The impact this disease may have on research using mice remains uncertain. The high incidence of this disease in the N:NIH(S)-B makes this strain an ideal study subject. Hepatitis in this strain may serve as a useful mode to study the pathogenesis of hepatitis in animals and humans.

Proposed Course:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-RR-10432-02 VR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenicity of CAR Bacillus (mouse origin) in Immunodeficient Strains of Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael Eckhaus, V.M.D.	Pathology Unit Chief	LSS, SSB, VRP, NCRR
Thomas Spencer, B.S.	Microbiologist	LSS, SSB, VRP, NCRR
Elaine Lamirande, B.S.	Biologist	LSS, SSB, VRP, NCRR
Annie Merriweather, B.S.	Histotechnologist	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda Md, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study was undertaken to determine the extent and severity of respiratory disease caused by CAR bacillus in immunodeficient mouse strains. Mice were inoculated intranasally with a standard number of CAR bacilli. These mice were killed at 2,4,8, and 16 weeks post inoculation. Tissues were obtained to determine the extent and severity of lesions by histopathological examination. The in vivo portion of the pathogenesis study has been completed and histopathologic analyses are in progress. The initial transmission study has been completed and histopathologic analyses are in progress. An additional transmission study using Balb/C mice has been initiated.

PHS 6040 (Rev. 5/92)

Objectives:

To evaluate the extent and severity of respiratory disease caused by CAR bacillus in several common immunodeficient mouse strains used in research worldwide, and to investigate the transmissibility of this agent.

Methodology:

Pathogenesis Study

A total of 25 mice each of the following strains: Balb/C, CB17 scid, nu/nu and bg-nu-xid mice were inoculated intranasally with 0.02 ml of allantoic fluid containing a standardized number of CAR bacillus organisms. Five mice per strain were inoculated with normal allantoic fluid as negative controls. Five inoculated mice and one negative control mouse per strain were sacrificed at the following time points: 2 weeks, 4 weeks, 8 weeks, and 16 weeks. Immunocompetent mice were anesthetized with metofane and bled to collect serum prior to euthanasia. Tracheal washes were performed at sacrifice on all animals and examined for the presence/absence of CAR bacillus. The trachea, lungs, and nasal turbinates were collected and fixed in 10% neutral buffered formalin for histologic evaluation.

Transmission Study

Five mice per strain were inoculated with CAR bacillus allantoic fluid as described above and housed in a Horsfall units. A cage of five additional mice of the same strain and a cage of Balb/C mice were housed adjacent to the inoculated mice in the same Horsfall unit. The Balb/C mice were survival bled peri-orbitally under metofane anesthesia at the following time points: 4 weeks, 8 weeks, 12 weeks, 16 weeks, 20 weeks, and 24 weeks to evaluate antibody titers to CAR bacillus. At 27 weeks all of the mice were sacrificed. The tracheas, lungs and nasal turbinates were collected in 10% neutral buffered formalin for histologic evaluation.

Major Findings:

Pathogenesis Study: Mice inoculated with CAR bacillus developed bronchopneumonia, bronchiectasis, and otitis media. The lesions were most severe in the Balb/C mice, comparable in severity in the CB17 scid and nu/nu, and least severe in the bg-nu-xid mice.

Transmission study: Serologic analyses confirmed aerosol transmission of the bacillus and infection of immunocompetent Balb/C mice housed in two of the four exposed cages. Histopathologic analyses of collected tissues are in progress. An additional transmission study using Balb/C mice has been initiated to further study transmission of CAR bacillus in a highly susceptible immunocompetent mouse strain.

Significance:

The results of the pathogenesis study indicate that while immunocompromised mice develop significant lesions due to infection with CAR bacillus, a competent immune system contributes to the overall severity of disease. Initial results of transmission studies confirms the possibility of aerosol transmission from one cage to another.

Proposed Course:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10433-02 VR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Detection and Diagnosis of Mycobacterial Infection in Nonhuman Primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ali A. Azadegan, D.V.M., Ph.D	Clinical Veterinarian Microbiologist	GRS, SSB, VRP, NCRR
Myrna G. Mandel, Ph.D.	Molecular Biologist	LSS, SSB, VRP, NCRR
Toni O,Neill, B.S.	Biologist	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

Tulane Regional Primate Research Center (RPRC)

LAB/BRANCH

Veterinary Resources Program

SECTION

Genetic Resources Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, Md, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The polymerase chain reaction (PCR) is a rapid, reproducible and reliable method of detecting specific sequences of DNA. The sensitivity of PCR to various strains of mycobacterium depends on the degree to which the target sequence is conversed in the organism. The specificity depends on the extent to which the target sequence is unique to the organism. The objective of the study is to develop a rapid and specific laboratory test for detecting and identifying mycobacteria within clinical samples from nonhuman primates. Published genus-specific primers have been used to synthesize probes in order to detect and differentiate the major pathogenic mycobacterial species. This was done in order to amplify the dnaJ genes from a broad spectrum of mycobacterial species. We and investigators at Tulane RPRC have analyzed two sets of blind coded bronchoalveole lavage, stomach lavage, urine and rectal swab samples for mycobacterial antigens. These samples were obtained from a group of NIH nonhuman primates housed at the Tulane RPRC. The two sets of samples were obtained from the animals at an interval of about 6 months apart. The Tulane RPRC investigators used a different mycobacterial probe. Upon analysis of the data from the two sets of blind coded samples both groups identified a mycobacterial antigen in the same animals at each sampling period. This group of animals is being studied by additional sampling over time. In additional samples obtained from nonhuman primates with known cases of tuberculosis have been collected and will be tested determine specificity and sensitivity of the probes. These results indicate that the polymerase chain reaction with genus-specific oligonucleotide probes may be useful for diagnosis of nonhuman primate mycobacterial infection.

Objective:

To develop a rapid, reproducible, reliable and specific laboratory test for detecting and identifying mycobacterial infections in nonhuman primates.

Methodology:

Bronchoalveolar lavage, stomach lavage, blood, urine and rectal swabs were obtained from ketamine HCL anaesthetized animals. These will be treated appropriately resulting in a suspension from which genomic DNA will be isolated utilizing sodium dodecyl sulfate (SDS), ribonuclease A and proteinase K followed by phenol-chloroform extraction and ethanol precipitation. DNA will be amplified in the thermocycler according to standard procedures. PCR primers are prepared based on the nucleotide sequence of the dnaJ gene of *M. tuberculosis.* The labeled species-specific oligonucleotide sequences synthesized to probe PCR products will be prepared from four mycobacterial species on the basis of the individual dnaJ genes sequences differing by more than two bases from each other. Dot blot analyses will be used to evaluate the amplified dnaJ gene products from the four mycobacterial species and from clinical isolates by hybridizing samples with the labeled species-specific oligonucleotide probes. Resulting autoradiograms will provide permanent records of relatedness between different species and confirmation of the preliminary diagnosis.

Major Findings:

We and investigators at Tulane Regional Primate Research Center (RPRC) have analyzed two sets of blind coded sbronchoalveole lavage, stomach lavage, urine and rectal swab samples for mycobacterial antigens. These samples were obtained from a group of NIH nonhuman primates housed at the Tulane RPRC. The two sets of samples were obtained from the animals at an interval of about 6 months apart. The Tulane RPRC investigators used a different mycobacterial probe. Upon analysis of the data from the two sets of blind coded samples both groups identified a mycobacterial antigen in the same animals in each sampling period.

Significance:

Tuberculosis remains one of the most important zoonotic diseases of man and primates. Despite the constant effort to test and identify potentially exposed and infected primates in laboratory animal facilities, a percentage of infected animals are diagnosed in necropsy rooms. Definitive diagnosis of tuberculosis and nontuberculous infections is provided only by demonstration of the presence of mycobacteria in clinical specimens. Microscopic examination, which has been the basic method for the diagnosis of tuberculosis, requires a relatively large number of bacteria to be present in specimens. Culture confirmation is a sensitive method for detecting mycobacteria, but it takes 3 to 8 weeks and another 2 to 4 weeks for final identification by biochemical analyses. The results of the study indicate that the polymerase chain reaction with genus-specific primers that amplify the dnaJ genes and subsequent dot blot analysis with species-specific oligonucleotide probes may be useful for diagnosis of primate mycobacterial infection.

Proposed Course:

Continuation. This group of animals is being studied with additional samples over time. In additional, samples obtained from nonhuman primates with known cases of tuberculosis have been collected and will be tested in order to determine specificity and sensitivity of the probes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10434-02 VR

PERIOD COVERED

October 1, 1993 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Embryo Cryopreservation Procedures for Laboratory Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

William F. Rall, Ph.D	NRC Senior Research Associate	GRS, SSB, VRP, NCRR
Patricia M. Schmidt, M.S.	Physiologist	GRS, SSB, VRP, NCRR
Xi Lin, D.D.S.	Visiting Scientist	GRS, SSB, VRP, NCRR
Carl T. Hansen, Ph.D	Geneticist	GRS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Genetic Resource Section

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The NIH Animal Genetic Resource (NIHAGR) maintains many unique rat genotypes used as models of normal and disease processes in man. Effective management of this resource would be facilitated by the development of embryo cryopreservation procedures for long-term banking. In order to develop banks of cryopreserved germ plasm from laboratory rats, protocols for each step of the embryo banking and rederivation process must be established. These would include the preparation and mating of embryo donor females, embryo collection, cryopreservation, thawing, and embryo transfer. Previous studies suggest that superovulation procedures developed for laboratory mice are not as effective when applied to laboratory rats. The principal objective of this project is to evaluate treatment with an analog of gonadotropin releasing hormone (GnRH-a) as an alternative approach to synchronize estrus cycles for embryo collection and cryopreservation.

Objective:

The objectives of this study are to: 1) evaluate the efficacy of gonadotropin releasing hormone analogue (GnRH-a) for synchronizing the estrus cycle of adult female rats from selected NIH Animal Genetic Resource (NIHAGR) genotypes; 2) collect and cryopreserve embryos from treated and mated females; and 3) determine the viability of cryopreserved rat embryos.

Methodology:

Adult female rats from selected NIHAGR genotypes were injected with a gonadotropin releasing hormone analogue (GnRH-a) and paired 3 or 4 days later with a fertile male. Successful mating was confirmed the following morning by the presence of spermatozoa in vaginal washes. (Mating was not confirmed when females were paired in the building 14F barrier). Preimplantation stage embryos were collected from euthanized females 5 days after sperm was detected or, in the case of females in the building 14F barrier, 9 days following injection of GnRH-a. Embryos were cryopreserved by either conventional slow freezing or vitrification methods using glycerol as the cryoprotectant. Following storage in liquid nitrogen (-196°C), embryos were warmed and diluted out of the cryoprotectant solution. The viability of embryos was determined by continued development *in vitro* to expanded blastocysts. Individual animal pedigree records were maintained on the Genetic Resource Section animal database system for embryos cryopreserved for long-term banking.

Major Findings:

The practical application to NIHAGR genotypes was assessed in a preliminary trial with two inbred stains, INR/N and PSDO/N. Of a total of 48 pedigreed INR/N females, 36 (75%) yielded morula to blastocyst stage embryos following treatment with GnRH-a and mating with brothers. A total of 377 pedigreed INR/N embryos were cryopreserved for future use. Similarly, of 125 pedigreed PSDO/N females, 49 (40%) yielded a total of 541 embryos for cryopreservation. The lower level of success for PSDO/N rats was associated with: 1) a higher incidence of females with irregular or no reproductive cycles, 2) male infertility; and/or 3) behavior incompatibilities between some females and males.

A further practical test of these procedures was initiated for pedigreed embryo banking of NIHAGR genotypes in a manner similar to that currently employed for mouse embryo banking. A total of 93 pedigreed females from various congenic strains, inbred strains and outbred stocks were injected with GnRH-a and mated with males within the 14F barrier rooms. Females were separated from the males 3 days later and sent to the ECP laboratory. Nearly all treated females (89%) yielded embryos when their oviducts and uteri were flushed 5 days after pairing. Forty-seven females (51%) yielded a total of 425 embryos at the required developmental stages (8-cell to blastocyst) and were cryopreserved for future use. The level of efficiency of rat embryo banking was equal to of slightly higher than that of mouse embryo banking (averages: 4.6 embryos banked/treated female, 9.0 embryos banked/female banked).

Significance:

The synchronization of estrus cycles of rats using GnRH-a provides a convenient method for increasing the efficiency of embryo collection and cesarian rederivation for rat genotypes maintained by the NIHAGR. Embryo cryopreservation studies indicated that the standard slow freezing and vitrification procedures developed for mouse embryos are equally effective for rat embryos. The banking of pedigreed embryos from females treated in a manner analogous to that currently employed to prepare mice for embryo cryopreservation indicates the suitability of this approach. Embryo transfer studies assessing the *in vivo* viability of cryopreserved rat embryos were initiated.

Proposed Course:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10435-02 VR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A New Pathogenic Bacterium of the Haemophilus/Pasteurella Group in Hamsters.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jennifer A. Burris, D.V.M.	Senior Veterinary Pathologist	LSS, SSB, VRP, NCRR
Michael A. Eckhaus, V.M.D.	Pathology Unit Chief	LSS, SSB, VRP, NCRR
Georgina F. Miller, D.V.M.	Senior Veterinary Pathologist	LSS, SSB, VRP, NCRR
Elaine Woo, B.S.	Biologist	LSS, SSB, VRP, NCRR
Theresa Lawrence, Ph.D.	Microbiologist	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.04	0.02	0.02

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues X
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An unclassified member of the bacterial family Pasteurellaceae has been isolated from cases of enteritis, conjunctivitis, and abscessation in a colony of golden hamsters (Mesocricetus auratus). This bacterium is a member of the Haemophilus/Pasteurella group, based on preliminary biochemical testing. It may represent a new species, but additional studies such as DNA hybridization will help to define the taxonomic position.

Three golden hamster strains are maintained in the colony - N:Syr (Syrian), N:M70 (Murphy), and N:LSH/TrN. The Haemophilus/Pasteurella group bacterium has been isolated from all strains. Both clinically ill and healthy adult hamsters representing all strains have been examined for gross and microscopic abnormalities, identification of gastrointestinal parasites, characterization of intestinal microflora, and identification of pathogenic bacteria at sites of inflammation.

Syrian hamsters from outside the colony have been used to determine the relative pathogenicity of the Haemophilus/Pasteurella group bacterium. Various routes of inoculation and doses of bacteria have been combined with clinical observations and post-mortem studies. This unique bacterial species in the Haemophilus/Pasteurella group is widespread in the NIH hamster colony. It is associated with disease processes which may interfere with the research activities of the NIH scientists. This study will help define the lesions produced, extent of the disease in the colony, and will result in the development of appropriate techniques to rid the colony of the infection and thus improve the health of animals used by NIH scientists.

Objectives:

To classify the Haemophilus/Pasteurella group bacterium present in the hamster colony, to test the pathogenicity of this bacterium, to determine the extent of natural infection in the colony, and to identify spontaneous diseases associated with this bacterium in the colony.

Methodology:

Hamsters are housed in conventional facilities in open-top cages. Complete autopsies are performed, tissues are fixed in neutral buffered formalin and routinely processed for light microscopic examination. Standard culture techniques are employed. Isolation of the Haemophilus/Pasteurella group bacterium is enhanced by GN broth and blood agar in a CO_2 atmosphere. Bacterial suspensions for ocular, oral, subcutaneous, and intramuscular injections are standardized using the McFarland nephelometer technique. Bacterial suspensions for experimental injections were obtained from frozen subcultures originally from clinical material.

Major Findings:

The Haemophilus/Pasteurella group bacterium is common in the intestinal tract of all strains of hamsters in the colony, and is often the predominant flora. Occasional biochemical variants of the Haemophilus/Pasteurella group bacterium have been found. This bacterium has been associated with spontaneous cases of enteritis, conjunctivitis, metritis, pneumonia, wounds, and abscesses in the hamster colony. Oral and parenteral administration of this bacterium to immunocompetent young adult CR:Syr hamsters have not caused gut colonization or disease. This bacterium therefore appears to be opportunistic and may require other co-pathogens to produce infections. For example in the gastrointestinal tract, heavy infections with Giardia are associated with enteritis and overgrowth of the Haemophilus/Pasteurella group bacteria. In wounds and abscesses, other pathogenic bacteria have been detected along with the Haemophilus/Pasteurella group bacterium.

Significance:

This unique bacterial species in the Haemophilus/Pasteurella group is widespread in the NIH hamster colony. It is associated with disease processes which may interfere with the research activities of the NIH scientists. This study will help define the lesions produced, extent of the disease in the colony, and will result in the development of appropriate techniques to rid the colony of the infection and thus improve the health of animals used by NIH scientists.

Proposed Course:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10436-02 VR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

DNA Fingerprinting Inbred Rats and Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Myrna G. Mandel, Ph.D.	Molecular Biologist	LSS, SSB, VRP, NCRR
Toni O'Neill, B.S.	Biologist	LSS, SSB, VRP, NCRR
Carl Hansen, Ph.D.	Geneticist	GRS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

American University

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Science Section,SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, Md 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this study is to augment the genetic quality assurance program of biochemical analysis of markers with the more definitive DNA fingerprinting technique. This molecular biological tool is indispensable for resolving ambiguous genetic data. The application of the DNA fingerprinting technique will permit greater precision in detecting genetic contamination and mutation by discovering differences between closely related strain, substrains and congenic rats and mice. Tissue samples from tails and livers from over 200 inbred and congenic strains of rats and mice have been processed and DNA purified. DNA fingerprint profiles have been developed and verified for many closely related albino mice and rats and for many black mice. A database is being developed to allow rapid location of the appropriate fingerprints. As rapidly as possible DNA fingerprint profiles will be available for all animals tested in this facility.

Objective:

To supplement the variety of chemical techniques used to identify inheritable traits in inbred rodents with DNA fingerprinting in order to provide greater assurance of their genetic integrity.

Methodology:

Small pieces of tails and livers have been collected and homogenized from the various inbred and congenic strains of rats and mice that have been received for genetic monitoring. Following incubation with a detergent (SDS, sodium dodecyl sulfate) and a protease (proteinase K), the homogenized and degraded tissues were extracted several times with phenol/chloroform, incubated with ribonuclease (to degrade ribonucleic acid) and finally were precipitated in isopropanol. Purified DNA was then incubated with a restriction endonuclease that cut DNA fragments in a unique pattern, depending on where and how often a particular repetitive sequence, recognized by the enzyme occurred in the strain's DNA. The DNA digests were then run very slowly (to increase resolution) on large agarose gels, transferred to nylon via Southern blotting,and exposed,under appropriate hybridization conditions, to a multilocus, alkaline phosphatase- labeled probe that reacts with a chemiluminescent substrate producing a transient intermediate that degrades to yield a signal on a photographic film. If the probe exposed to the unknown DNA finds sequences to complement, it will hybridize-attach itself-forming a fingerprint on the film. Additional studies have been initiated to determine if radioactive labeled double stranded probes will give more reproducibility.

Major Findings:

It has been possible to distinguish between many closely related inbred rodent strains using DNA fingerprints. Tissue samples from tail and livers from over 200 inbred and congenic strains of rats and mice have been processed and the DNA purified. DNA fingerprint profiles have been developed and verified for many closely related albino mice and rats and for many black mice. A database is being developed to allow rapid location of the appropriate fingerprints. Data available on over 100 inbred rodent strains indicates that the DNA fingerprinting technique is more rapid and accurate in distinguishing closely related rodent strains than is the biochemical techniques. In addition, genetic anomalies in these closely related strains, that have been undetectable by the biochemical assays, have been identified.

Significance:

This molecular biology procedure can test for genetic integrity of live animals. Additionally, 1 film of 30 fingerprints eliminates the need for sacrificing and processing the tissue of 30 animals and performing as many as 8 biochemical assays. Development of a computer database for the DNA fingerprints will enhance our quality assurance capability.

Proposed Course:

Continuation. As rapidly as possible DNA fingerprint profiles will be available for all animals tested in this facility. Attempts will be made to correlate DNA fingerprint profiles with genetic change as applicable to transgenic and knockout animals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10437-02 VR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Infection with Mycoplasma pulmonis in SCID and BALB/C Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Georgina F. Miller, DVM	Staff Veterinary Pathologist	LSS, SSB, VRP, NCRR
Antoinette O'Neill, BS	Biologist	LSS, SSB, VRP, NCRR
David Madden, DVM	Chief, LSS	LSS, SSB, VRP, NCRR
Myrna Mandel, PhD	Supervisor, GMU	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

Diagnostic Laboratory Unit & Genetic Monitoring Unit, VRP

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mycoplasma pulmonis is one of the most important pathogens of laboratory rodents. It is the etiologic agent of Murine Chronic Respiratory Disease (CRD) which was ubiquitous in laboratory rats and mice into the 1970's. This disease has almost been eliminated. In research colonies, detection of subclinical infections by parasitic, bacterial, viral, and mycoplasmal agents generally depends on testing of designated sentinel animals. Because immunodeficient mice do not make antibodies, serologic tests, which would detect them, are of no value in these animals. However, the nearly universal practice of housing immunodeficient mice in microisolator cages effectively isolates the sentinels (who may be housed in the same rack but in different cages) from any exposure to pathogens harbored by the immunodeficient mice. The objective of this study is to 1) determine the gross and histopathologic changes produced by Mycoplasma pulmonis in immunodeficient mice (SCID) inoculated by both the respiratory and genital routes and compare them with those found in immunocompetent mice (BALB/C) under identical conditions; and 2) establish the reliability of polymerase chain reaction (PCR) techniques in detecting mycoplasmosis, and compare its sensitivity with that of routine culture. Mice will be infected by either the intranasal or intravaginal route. Infection will be detected by serology (as appropriate), culture, PCR, and by gross and histologic changes. Samples for mycoplasma culture and PCR will be collected from the oropharynx, trachea, lung, and uterus. The following tissues will be screened for histologic changes: nasal sinuses, middle ears, trachea, lungs, vagina and uterus.

OBJECTIVE:

The objectives are 1) to determine the gross and histopathologic changes produced by *Mycoplasma pulmonis* in immunodeficient mice (SCID) inoculated by both the respiratory and genital routes and compare them with those found in immunocompetent mice (BALB/C) under identical conditions and 2) to establish the reliability of polymerase chain reaction (PCR) techniques in detecting mycoplasmosis, and compare its sensitivity with that of routine culture.

METHODOLOGY:

The experiment will be conducted in 2 phases. Phase I will establish the optimal low and high does for the strain of *Mycoplasma pulmonis* used in this experiment. Phase 2 will compare pathologic changes, culture and PCR detection in 2 strains of mice: an immunocompetent strain (BALB/C) and an immunodeficient strain (Severe Combined Immunodeficient or SCID), using 2 infection routes (respiratory and genital), 2 dose levels, and 4 time points (3 days, 1 week, 3 weeks, and 8 weeks). Mice will be infected by either the intranasal or intravaginal route. Infection will be detected by serology (as appropriate), culture, PCR, and by gross and histologic changes. Samples for mycoplasma culture and PCR will be collected from the oropharynx, trachea, lung, and uterus. The following tissues will be screened for histologic changes: nasal sinuses, middle ears, trachea, lungs, vagina and uterus.

MAJOR FINDINGS:

Phase I has been completed; the optimal low and high doses of *M. pulmonis* were determined, the primers for PCR were selected, and the PCR test conditions optimized. The PCR has proved as reliable as culture in the detection of *M. pulmonis* infection.

SIGNIFICANCE:

This study will determine methodologies to allow more reliable diagnostic techniques for subclinical mycoplasmosis in immunodeficient mice, and prevent unrecognized subclinical infections from interfering with biomedical research. It will also compare sensitivity and reliability of PCR with routine culture, and assess the possibility of replacing time-consuming culture techniques with more rapid and cost-effective PCR.

PROPOSED COURSE:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-RR-10438-02 VR

October 1, 1993 to September 30, 1994

Hemosiderosis in Marmosets: A Potential Animal Model for Human Iron Overload

Georgina F. Miller, DVM   Staff Veterinary Pathologist   LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

Laboratory of Comparative Ethology, NICHD

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hepatic hemosiderosis is a commonly recognized finding in marmosets as well as other new world monkeys kept in zoologic, breeding and research colonies. Based on the absence of associated liver pathology or elevation in serum liver enzymes in affected animals, the condition has been considered to be of no clinical or pathologic significance. A high incidence of hepatic and systemic hemosiderosis was detected in marmosets from NIH's colony submitted for routine necropsy to the Pathology unit, LSS, SSB, VRP, NCRR. These animals were used in behavior research and had all died or were euthanized due to spontaneous disease or trauma. The animals were fed a routine diet and were not given experimental drugs.

Most marmosets were debilitated and cachectic at the time of death. Death was caused by a variety of conditions, with underlying, predisposing factors not always identified. The objective of this study is to determine the incidence and distribution of hemosiderosis in a marmoset (Callithrix jacchus) colony maintained at NIH, judge its potential impact on animal health, and compare its pathogenesis and tissue distribution to human iron-overload conditions. In this retrospective study, necropsy records and microscopic tissue sections from 46 marmosets presented to the pathology unit for necropsy between 1989 and 1992 were examined. Marmosets were excluded from the study only if no archived tissues were available for review. Hemosiderosis was found in 95% of the marmosets studied. The significance of this may allow development of novel diagnostic and prophylactic regimes applicable to humans. The pattern is comparable to that found in human hereditary hemochromatosis and differs from that found in nutritional and transfusion-induced hemosiderosis.

OBJECTIVE:

To determine the incidence and distribution of hemosiderosis in NIH's marmoset colony, judge its potential impact on animal health, and compare its pathogenesis and tissue distribution to human iron-overload conditions.

METHODOLOGY:

In this retrospective study, necropsy records and microscopic tissue sections from 46 marmosets presented to the pathology unit for necropsy between 1989 and 1992 were examined. Marmosets were excluded from the study only if no archived tissues were available for review. The study included common marmosets (*Callithrix jacchus*) and pygmy marmosets (*Cebuella sp.*) who ranged in age from 2 days to aged adults. Sections of liver from all animals were examined histologically, using routine H&E, Prussian blue stain for iron, and Masson's trichrome for collagen. Each sections was judged as to the degree of hepatocellular hemosiderosis, Kupffer cell hemosiderosis, and perisinusoidal fibrosis. Hemosiderosis was also evaluated in other organs available for review. Necropsy reports were reviewed to determine nutritional status at time of death, the cause of death, and secondary findings.

MAJOR FINDINGS:

Hepatic hemosiderosis was found in 100% of the marmosets included in the study; 100% of the neonatal marmosets had stainable iron in the liver. Hemosiderin accumulation was confined to hepatocytes in 45% of the animals; in the remainder, the hemosiderin accumulated in both hepatocytes and Kupffer cells. Hepatic hemosiderosis increased in severity with age, and was more severe in males. Hepatic perisinusoidal fibrosis, indicative of a toxic insult to the liver, was found in 32% of the marmosets with mild to severe hepatocellular fibrosis. Dehydration and inanition (absence of body fat) were noted at necropsy in the majority of animals. The causes of death were varied, and frequently were undetermined. Several animals died with terminal septicemia and bacteremias, suggestive of a state of immunosuppression. Anemia was found in only 1 animal. A manuscript reporting these findings has been submitted for publication.

SIGNIFICANCE:

The pattern of iron accumulation in the liver of marmosets shares features of both human hereditary hemochromatosis and human nutritional iron overload. Iron accumulation in marmosets appears associated with a state of terminal debility. While this retrospective study could not determine the cause of hepatic hemosiderosis in the marmoset, evidence suggests that the standard marmoset diet fed to these animals contains excess iron, and the marmoset is unable to adequately regulate iron absorption of dietary iron. The defective mechanism leading to excess iron absorption in the marmoset may be similar to the defect in human hereditary hemochromatosis; the marmoset is potentially a valuable animal model for this common human inherited condition. A spontaneous animal model for hemochromatosis would allow researchers to manipulate dietary, drug, and phlebotomy therapies in ways not possible with the current experimentally induced models. Additionally, identification of gene sequences and molecular pathways responsible for iron accumulation in the marmoset may allow development of novel diagnostic and prophylactic regimes applicable to humans. The findings also suggest that hepatic hemosiderosis is an important cause of debility and shortened life span in captive marmoset colonies which may be controlled by adjusting the iron content in the diet.

PROPOSED COURSE:

Completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10439-02 VR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Environment on Socially-Housed Rhesus Reactions to Novel Objects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kathryn A.L. Bayne, M.S., Ph.D., D.V.M.	Chief, BNU	LSS, SSB, VRP, NCRR
Elizabeth E. Hill, B.S.	Biologist, BNU	LSS, SSB, VRP, NCRR
Corrine Lutz, B.S.	Biologist, LNP	NIMH

COOPERATING UNITS *(if any)*

Laboratory of Neurophysiology, NIMH

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.5	.1	.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this study is to address the influence of environmental complexity on the primate's reactions to novel objects. Two separate housing facilities were used for the study: one with a typical enrichment program and one with a more complex and diverse program. Eight rhesus monkeys (Macaca mulatta) housed in pairs were used for the study. An A-B-A paradigm was used. Behavioral data were collected. Each animal was observed 15 minutes a day, 3 days a week for two weeks prior to receiving a novel object in or on the cage. They were then observed for the same amount of time with a novel object (mirror, wishbone shaped dog toy, split PVC pipe, knotted dog bone). The object was changed every two weeks, for a total of 8 weeks. Finally, they were observed for 2 weeks following the removal of the last novel object. Preliminary results show a difference in response to device utilization in different facilities. Additionally, this research has shown that nonhuman primates respond as individuals to the addition of novel objects to their environments. It was also noted that the subjects continued to utilize the standard enrichment devices that were provided as a constant throughout the study.

PHS 6040 (Rev. 5/92)

Objective:

To address the influence of environmental complexity on the primate's reactions to novel objects.

Methodology:

Two separate housing facilities were used for the study: one with a typical enrichment program and one with a more complex and diverse program. Eight rhesus monkeys (Macaca mulatta) housed in pairs were used for the study. An A-B-A paradigm was used. Each animal was observed 15 minutes a day, 3 days a week for two weeks prior to receiving a novel object in or on the cage. They were then observed for the same amount of time with a novel object (mirror, wishbone shaped dog toy, split PVC pipe, knotted dog bone). The object was changed every two weeks, for a total of 8 weeks. Finally, they were observed for 2 weeks following the removal of the last novel object. The behavioral data was collected using a barcode behavioral recording program and a Tandy laptop 102 computer. Interobserver reliability averaged 95%.

Major Findings:

Results appear to show a difference in response to device utilization in different facilities. Additionally, this research has shown that nonhuman primates respond as individuals to the addition of novel objects to their environments. It was also noted that the subjects continued to utilize the standard enrichment devices that were provided as a constant throughout the study. Further statistical analysis is ongoing.

Significance:

The novelty effect of an enrichment device is important relative to animal husbandry and the optimal schedule for changes of enrichment devices. Previous studies investigating the topic of environmental complexity have dealt mainly with the neophobic response of rodents to novel foods or liquids. The literature concerning primate neotic responses reveals that monkeys reared in isolation exhibit more fear of unfamiliar objects than do feral and mother-peer reared and that when exposed to a series of novel objects.

Proposed Course:

Completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10442-01 VR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hepatic Hemosiderosis in Marmosets

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Georgina F. Miller, DVM	Staff Veterinary Pathologist	LSS, SSB, VRP, NCRR
Dennis Barnard, MS	Nutritionist	LSS, SSB, VRP, NCRR
Ruth Woodward, DVM	Clinical Veterinarian	PUP, RAB, VRP, NCRR
Bernard Flynn, DVM	Chief, Primate Unit/Poolesville	PUP, RAB, VRP, NCRR

COOPERATING UNITS *(if any)*

Behavior and Nutrition Unit, LSS, SSB and Primate Unit/Poolesville, PS, RAB.

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hepatic hemosiderosis is a commonly recognized finding in marmosets as well as other new world monkeys kept in zoologic, breeding and research colonies. Based on the absence of associated liver pathology or elevation in serum liver enzymes in affected animals, the condition has been considered to be of no clinical or pathologic significance. A high incidence of hepatic and systemic hemosiderosis was verified in a retrospective study of marmosets from NIH's colony submitted for routine necropsy. These animals had been used in behavior research and had died or were euthanized due to spontaneous disease or trauma. There was no history of experimental diet or drug administration in these animals. At death, many of the adults were in poor body condition due to undetermined causes.

OBJECTIVE:

To establish the role of dietary iron in the development of hepatic hemosiderosis in the common marmoset, *Callithrix jacchus*, to document the health affects of this condition as well as possible health benefits of a low iron diet, and to assess the ability of blood chemistries to diagnose iron overload.

METHODOLOGY:

A colony of young adult common marmosets are divided into 2 groups, and fed nutritionally complete diets which differ only in their iron content. The low dietary iron group receives a diet containing less than 100 ppm iron, while the high dietary iron group receives a diet containing 500 ppm iron. Baseline blood work, including serum iron and transferrin levels, and liver biopsies for iron analysis were performed prior to beginning the test diets. Hematology and serum chemistries will be performed every 6 months, with liver biopsies and iron analysis performed every 12 months. Animals will be weighed and assessed for health status at regular intervals. A complete necropsy will be performed on any animal dying during the study.

MAJOR FINDINGS:

The baseline liver iron content of these 2-3 year old common marmosets ranged from 1136 to 6182 micrograms/gram, dry weight. While normal liver iron content for marmosets is unknown, up to 1800 micrograms/gram is considered normal in humans. The marmosets have accepted the test diets.

SIGNIFICANCE:

1. If iron overload causes chronic debility in marmosets as it does in humans, and iron overload can be prevented by feeding marmosets a low iron diet, then many of the poorly understood debilitating conditions that plague marmoset colonies may be prevented. The results of this study could significantly improve the health of marmosets in research laboratories, zoologic parks and breeding colonies world-wide, and permit more extensive use of these small primates in biomedical research.

2. Iron overload is an important cause of human disease, which if untreated, can be fatal. To date, an adequate animal model has not been identified. Marmoset hepatic hemosiderosis is similar to human hereditary hemochromatosis (the most common cause of human iron overload in North America and Europe) in many key aspects. If successful, this study will result in the development of two stable well-characterized marmoset populations, one with hemosiderosis and one without. These animals would be available for testing therapeutic and prophylactic regimens potentially applicable to humans, and for exploring underlying pathogenic mechanisms of iron overload.

PROPOSED COURSE:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-RR-10453-01 VR

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Comparative Behavioral Analysis of Vertical Cage Space for Group Housed Rhesus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kathryn A.L. Bayne, M.S., Ph.D., D.V.M.	Chief, BNU	LSS, SSB, VRP, NCRR
Sandra L. Dexter, B.S.	Biologist, BNU	LSS, SSB, VRP, NCRR
Katherine P. Weld, M.S.	Biologist, BNU	LSS, SSB, VRP, NCRR
Gretchen M. Strange, M.S.	Biologist, BNU	LSS, SSB, VRP, NCRR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Veterinary Resources Program

SECTION
Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION
NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	.1	.9

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Guide for the Care and Use of Laboratory Animals recommends that socially housed Group 3 primates be provided a cage with a minimum of 72" in height. The purpose of this study was to determine what effect varying cage height has on the behavior or use of vertical space by socially housed rhesus monkeys (*Macaca mulatta*). A variety of behaviors, as well as vertical cage space utilization, were recorded for each subject in a cage equipped with a perch and one without a perch. Utilizing an adjustable ceiling, behaviors manifested by the subjects under eight different cage heights ranging from 30" to 72" were examined. Animals were observed for 20 minutes, three times a week for a two week period at the assigned height. Results indicate that frequency and duration of several behaviors were significantly effected by cage height changes. Further data analysis will test for sex differences as well as if the addition of a perch effected utilization of the cage space.

Objective:

To study the effects of varying cage height for group-housed rhesus monkeys from 30" (minimum cage height required by the Guide for individually housed Group 3 animals) to 72" (minimum height required by Guide for animals grouped in pens) has on the behavioral profile or the use of the cage.

Methodology:

Cage height is varied by utilizing an adjustable ceiling to produce eight different cage heights (72", 66", 60", 54" 48", 42" 36" and 30"). A focal animal sampling technique was used to gather data on each individual in the cage. The subjects were observed for frequency and duration of several categories of behavior: cage-directed, stereotypic, self-directed, repetitive locomotion, self-groom, social groom, time spent at the top of the cage, time spent at the bottom of the cage and frequency of time spent at the middle of the cage. Additionally, a comparison was made between the cage without a perch and a cage with a perch to evaluate possible inherent biases in space use due to the presence of "cage furniture".

Major Findings:

Results indicate that frequency and duration of several behaviors were significantly effected by cage height changes. Further analysis will test for sex differences as well as if the addition of a perch effected utilization of the cage space.

Significance:

Under the new amendments to the Animal Welfare Act (1991), social housing of non-human primates as a means of enhancing their "psychological well-being" is being considered by the U.S. Department of Agriculture the default method of housing. In many cases, NIH investigators have requested that small social groups of nonhuman primates be housed in large cages or pens. According to the Guide: "Space in group cages should be enriched with structures such as resting perches and shelters. The minimum height of pens and runs used to house nonhuman primates should be 6 ft (1.8 m)". The Veterinary Resources Program currently makes group pens available in nonhuman primate holding facilities. However, due to the height of the animal room door frames, group cages that meet the 6 ft Guide requirement will not fit through the doors into the rooms. Since alteration to the door frames would require a costly and extensive renovation of the facility, strict adherence to the Guide would prohibit group housing pens.

More data on the consequences of alterations in available cage space are particularly essential at this time, since the NIH and the Institute of Laboratory Animal Resources (ILAR) are proposing to write an updated draft of the Guide for the Care and Use of Laboratory Animals.

Proposed Course:

Completed.

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